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THE ANTIBACTERIAL EFFECT OF SURFACTANTS IN COMBINATION WITH A COMMONLY USED PHARMACEUTICAL PRESERVATIVE AGAINST BACTERIAL CONTAMINANTS

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ABSTRACT

The ability of the microbial contaminant to cause undesirable changes on the pharmaceutical product has prompted the search for suitable antimicrobial agents. Such agent must be able to cope with the contaminants encountered during manufacture, storage and use of the products. It is therefore important that the efficacy of these agents and their spectra of activity be established before their use. In this study, the effect of two surfactants (cetrimide or sodium lauryl sulphate) on the activities of a commonly used pharmaceutical preservative (methylparaben) against bacteria contaminants were evaluated. Forty brands of used topical creams were selected for the isolation of microbial contaminant and subjected to antimicrobial susceptibility testing. This involves the determination of the minimum inhibitory and bactericidal concentration as well as the evaluation of combined effects of preservative and surfactants using continuous variation checkerboard method. Results showed that both surfactants and preservative had antimicrobial effects. Amongst all three agents, cetrimide exhibited the most bactericidal activity against the 4 isolates tested. The combined effect of methylparaben and two surfactants showed that majority of the combination exhibited synergism, a few showed additivity, and none demonstrated antagonism against test organisms. This result highlights the role of surfactants in increasing the antimicrobial activity of the preservative. This would probably be due to the inherent ability of some surfactants, to sensitize the bacteria cells and increase their permeability to the actions of the antimicrobial agents. The study would

therefore be useful in selection and optimization of tolerable dosage for pharmaceutical preparations.

KEYWORDS: Antibacterial, Surfactants, Pharmaceutical, Methylparaben, Checkerboard, Creams.

INTRODUCTION

Antimicrobial preservatives are included in pharmaceuticals products to prevent and control the growth of microorganisms from contamination during manufacture, storage or consumer use.[1, 2] Microbial contaminants of concern include pathogens, opportunistic pathogens and non-pathogenic microorganisms which can alter the chemical or physical properties of a product resulting in phase separation, discoloration, formation of gas or off odours, change in the pH of the formulation. Ideally, preservatives should contain various combinations and amounts of chemical biocides to provide a broad spectrum of activity.[3] Commonly used preservatives include the parabens, imidazolidinyl urea, quaterium-15 formaldehyde solution and isothiazolinones. Most of preservatives offer a high antimicrobial efficacy, but many have the potential to cause adverse reactions on sensitive skin.[4-6] Surfactants on the other hand are widely used in personal care products as emulsifiers, solubilizers, wetting and cleaning agents, foam producers and conditioning aids. Among them, cationic surfactants such as quaternary ammonium compounds are known to exert excellent antimicrobial activity against both Gram-positive and Gram-negative bacteria and are the most useful antiseptics and disinfectants.[7]

In the evaluation of antimicrobial combinations against microorganisms, there are laid-down criteria's for determining the level of success of the antimicrobial combination. The overall goal in the use of preservative combinations will be based on the confirmation of the presence of synergism.[8, 9] It means that the inhibitory or antimicrobial effect of the combination will be greater than the arithmetic summation of the effects of the single individual agents.[9, 10] The selection of an appropriate combination requires an understanding of the potential for interaction between the antimicrobial agents and their individual mechanisms of action as previously reported.[11]

A few authors have reported on the evaluation of antimicrobial preservative combinations. Some of which includes the monomeric method for the evaluation of preservatives by O' Callaghan and Kerry[12] The author examined a combination of 5 agents (nanoparticled

solubilisates – sorbic acid, benzoic acid and rosemary extract, and non-nanoparticled chitosans – of two different molecular weights) against cultures derived from cheese. They found that the top-performing antimicrobials contained chitosan and/or rosemary, individually or in combination which could be use as active agents in cheese packaging.[12] Patrone *et al*.[7] investigated the synergistic activities of essential oil and/or surfactants in combination with cosmetic preservatives against two organisms. Their study tries to evaluate possible synergistic antimicrobial interactions between common cosmetic preservatives and selected essential oils or surfactants. Their antimicrobial efficacy of the agents was tested against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using broth micro-dilution assay. Synergy was observed when essential oils of eucalyptus and mint were combined with methylparaben against *P. aeruginosa*, while essential oils of mint, oregano and sage combined with propylparaben and imidazolidinyl urea acted against *S. aureus*.[7]

A related study by Jackson *et al*.[11] showed that in vitro tests on combination of *Euphorbia hirta leaf* extract and Nystatin at a given ratio has a possible clinical significance in the treatment of fungal infection caused by *Candida albicans*. Although some combination ratios resulted in synergistic effect, while others indicated indifferent activities against the test organism. They concluded that the selection of an appropriate combination requires an understanding of the potential for interaction between the antimicrobial agents and their individual mechanisms of action. Orhan *et al*.[13] used two different synergy testing methods, the checkerboard and the E test methods to compare the in vitro efficacies of various antimicrobial combinations against 16 *Brucella melitensis* strains isolated from blood cultures. The rate of agreement of the E test and checkerboard methods was found to be 55 %. The most significant results were the combinations between streptomycin and doxycycline in which four strains showed synergistic activity using E test, antagonistic activity with the checkerboard method and one strain showed antagonistic activity with both methods. White *et al*.[14] compared the checkerboard method, the time-kill method, and E test to investigate the effects of four different antibiotic combinations against *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* strains. There was 44 to 88 % agreement between the time-kill and checkerboard methods and 63 to 75 % agreement between the time-kill and E test methods. The rate of agreement between the checkerboard and E test methods was found to be 75%. Dundar and Otkun[15] investigated the Synergistic efficacies of ceftazidime-tobramycin, piperacillin/tazobactam-tobramycin, imipenem-tobramycin, imipenem-isepamycin, imipenem-ciprofloxacin and ciprofloxacin-tobramycin

combinations by checkerboard technique in 12 multiple-resistant and 13 susceptible *P. aeruginosa* strains. The result of their work shows the ratios of synergy in ceftazidime-tobramycin and piperacillin/tazobactam-tobramycin combinations as 67 %, and 50 %, respectively. The ratio of synergy in ceftazidime-tobramycin, piperacillin/tazobactam-tobramycin, imipenem-tobramycin, imipenem-ciprofloxacin and imipenem-isepamycin combinations were 31 %, 46 %, 15 %, 8 %, 8 % respectively in susceptible strains, whereas synergy was not detected in ciprofloxacin-tobramycin combination. Antagonism was not observed in any of the combinations. They also concluded that although the synergistic ratios were high in combinations with ceftazidime or piperacillin/tazobactam and tobramycin, the concentrations in these combinations did not reach the usual clinically available levels.[15]

This concept of synergism was also described by Sueke *et al*.[16] on *in vitro* investigation of synergy or antagonism between antimicrobial combinations against isolates from *S. aureus* and *P. aeruginosa*. In their work, they observed that the combinations meropenem and ciprofloxacin; meropenem and telcoplanin; moxifloxacin and tecloplanin, and ciprofloxacin and tecloplanin gave the highest synergy of about 60-80% for Staphylococcus aureus while the meropenem/ciprofloxacin combination gave the lowest mean FIC for Pseudomonas aeruginosa isolates, with 90% showing synergistic effect.

Although series of investigations have been carried out on the combination of two or more antimicrobials as an important method for, at least, delaying the emergence of resistance.[11, 17] Yet there are still concerns about the continuous emergence of bacterial strains that are resistant to conventional preservatives especially in the developing countries. There is the need for a continuous search for alternative preservative systems that would cope with the contaminant encountered during manufacture, storage and use of the products. Much attention has been focused on exploiting the antimicrobial potential of multifunctional ingredients which are not added to formulations primarily for their antimicrobial activity, but which may contribute to product preservation.[7]

There seems to be limited material on the effect of surfactants (cetrimide or sodium lauryl sulphate) on the activities of a commonly used pharmaceutical preservative (methylparaben) against bacteria contaminants. This research is embarked on with the aim of determining the combined effect of the antimicrobial agents in terms of the magnitude of inhibition as compared with the single agents. Related literature will generally serve as a background towards understanding the concept behind preservative-surfactant combinations in

pharmaceutical preparations. This study will therefore be of great significance to pharmaceutical companies in general as it will go a long way in explaining the rationale behind the use of preservative combinations in the preservation of pharmaceutical products and the techniques which can be adopted in their evaluation

MATERIALS AND METHODS

Antimicrobial agents

Antimicrobial agents used in this study were Sodium lauryl sulfate (KERMEL), Cetrimide (Fluka AG, Buchs SG), Methylparaben (KEM light). They were used with minimum handling to avoid possible contamination.

Culture Media

The culture media used in this study were Nutrient agar/broth (Lab M), Plate count agar (Lab M), Mueller Hinton broth (Oxoid), MacConkey agar (Lab M), Mannitol salt agar (Lab M), Cetrimide agar (Lab M). They were reconstituted according to manufacturers' instructions and used for the isolation, cultivation, and maintenance of a variety of fastidious and non-fastidious microorganisms.[18-20]

Cultivation of Microorganisms

The cultivation of microorganisms began with the formation/preparation of a homogenate in the form of a 10 % solution or dilution to isolate a pure culture for the identification/characterization of the microbial isolate obtained from the mixed culture. Other process involved were the evaluation of the microbial content in the sample, all of which are presented below:

Preparation of Homogenate

Liquids - 1 mililitre of the liquid was decimally diluted into a 9 mL Muller Hinton broth (MLB) in 20 × 150 mm sterile capped tubes for a 10^{-1} dilution.[21]

Cream and Oil-based products - 1 gram of the sample was aseptically dispensed and weighed into sterile capped tube containing 1 mL sterile Ringers solution. The volume (total) was then adjusted to 10 mL with sterile nutrient broth for a 10^{-1} dilution as previously described.[21]

Isolation and Identification of Microbial Isolates

The test organisms were isolated from isolated from used topical cream samples. Identification of the bacterial isolates were performed according to standard bacteriological techniques previously described.[19, 22] All isolates were sub-cultured onto selected culturing media and maintained at – 4 ^{o}C until ready for use.[23]

Susceptibility Testing

Preparation of culture suspension

Stock cultures of the isolated organisms obtained from the screened samples were used for the preparation of culture suspension. The bacterial inoculums were prepared with 2-3 hour broth culture of each isolate, adjusted to a turbidity equivalent to 0.5 McFarland Standard.

Preparation of antimicrobial agent stock solution

Appropriate quantities of the powdered preservatives were weighed and constituted with sterile distilled water to form the required concentration. For example,

- *Sodium lauryl sulfate*: 1 gram of the powder was weighed out and constituted with 50 mL of sterile distilled water to form a stock solution of 20 mg/mL.
- *Cetrimide*: 1 gram of the powder was constituted with 50 mL of sterile distilled water to form a stock solution of 20 mg/mL.
- *Methylparaben*: l gram of the solution was diluted in 50 mL of sterile distilled water to make up to 50 mL of the stock solution of 20 mg/mL.

The sensitivity of test organisms to the antimicrobial agents was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the agents using the two-fold broth dilution and agar dilution techniques respectively as recommended by the Clinical Laboratory Standard Institute.[24]

Determination of Minimum Inhibitory Concentration (MIC)

8 sterile capped tubes were used for the determination of the minimum inhibitory concentration. The first 6 tubes were labelled 1-6, while the last two tubes were labelled A and B respectively. The steps include:

- 2 mL each of sterile double strength Mueller Hinton broth was dispensed into the sterile capped tubes.

- Into test tube 1 was added 2 mL of the already prepared stock solution of the test agent (known concentration) and the mixture thoroughly mixed to obtain half the concentration of the stock solution.
- A volume of 2 mL was then transferred from tube 1 to tube 2 and mixed to produce a solution with half the concentration of tube 1.
- This process was repeated sequentially for tubes 3, 4, 5 and 6.
- Then 2 mL of the mixture in tube 6 was pipetted into tube A which served as a control for transference sterility.
- Finally, to each tube except tube A was added 0.1 mL of the standardized bacterial culture and incubated within 20 minutes of adding the inoculums for 24 hours at a temperature of 37 ^{o}C.

Determination of minimum bactericidal concentration (MBC)

After the determination of the minimum inhibitory concentration, a loopful of all the contents of the tubes that did not show visible growth was streaked on a nutrient agar plates. The plates were incubated for 48 hours at 37 ^{o}C after which the minimum bactericidal concentration was noted.[24, 25]

Evaluation of Combined Effects of Preservative and Surfactants

After the determination of the MIC and MBC, the preservative (methylparaben) was separately combined with cetrimide (cationic surfactant) and sodium lauryl sulfate (anionic surfactant) to evaluate their effect or activity against the same test microorganisms as previously described.[8, 25-29] Equal volume of double strength Mueller Hinton broth and the required concentration (i.e. MIC^{2}) of each agent and were prepared and sterilized prior to their combination. The antimicrobial agents were then combined in a binary form ratio (0 mL + 5 mL; 1 mL + 4 mL; 2 mL +3 mL; 3 mL + 2 mL; 4 mL + 1 mL; 5 mL + 0 mL of preservative and surfactant respectively). The resulting solutions were then dispensed into test tubes containing sterile double strength Mueller Hinton broth to give serial two-fold dilutions. Bacterial suspensions with a turbidity corresponding to 0.5 McFarland standard were prepared to yield a final inoculum of approximately 3 - 5 $\times$ 10^{5} CFU mL^{-1}. Growth and sterility controls were included for each proportion. Each test tube was inoculated with 0.1 mL of test microorganism and then incubated for 24 hours at 37 °C. Fractional Inhibitory Concentration (FIC) for each proportional mixture was calculated and analysed for

synergism, additivity, indifference and antagonism using the formula in equations 1 to 3 below.[26, 27, 30]

$$FIC_{index} = FIC_{preservative} + FIC_{surfactant} \quad \text{Equation 1}$$

$$FIC_{preservative} = \frac{\text{MIC of preservative in combination with surfactant}}{\text{MIC of preservative alone}} \quad \text{Equation 2}$$

$$FIC_{surfactant} = \frac{\text{MIC of surfactant in combination with preservative}}{\text{MIC of surfactant alone}} \quad \text{Equation 3}$$

RESULTS AND DISCUSSION

As shown in Fig. 1, no more than four bacterial species were isolated from 19 out of 40 samples screened and identified as previously described.[19] Their prevalence was in the following order: *Staphylococcus epidermidis* (39 %) *Staphylococcus aureus* (34 %), *Escherichia coli* (22 %), *Klebsiella pneumoniae* (5 %). The results of the isolation from samples simply agrees with literature reports which indicates that cosmetic and topical products need not be sterile but may contain low levels of microbial load before use.[31] Also, the warm and rather humid climatic conditions that prevail in most tropical countries, including Nigeria, would tend to support the survival and growth of many microorganisms.[22] In a situation whereby a nutritionally rich pharmaceutical/cosmetic product is already in use, rapid growth and multiplication would be expected.

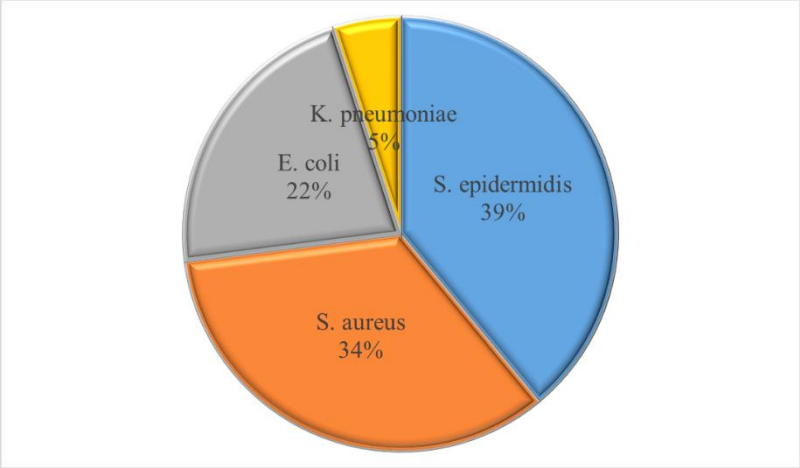

Fig. 1: Prevalence of the microbial isolate from used topical cream samples.

Susceptibility Test

The susceptibility of test microorganisms to the antimicrobial agents were evaluated by determining the minimum inhibitory concentration (MIC) of the agents using the two-fold broth dilution technique as recommended by the Clinical Laboratory Standard Institute (CLSI) and the result of the susceptibility test shown in Fig. 2 and 3. The lowest concentration of antimicrobial required to kill a bacterium was measured and this value is referred to as the minimum bactericidal concentration (MBC) or minimum lethal concentration (MLC). To determine the MBC, agar dilution system was employed. MBC is the concentration of antimicrobial that kills at least 99.9 % of the original bacterial inoculum as previously reported.[32]

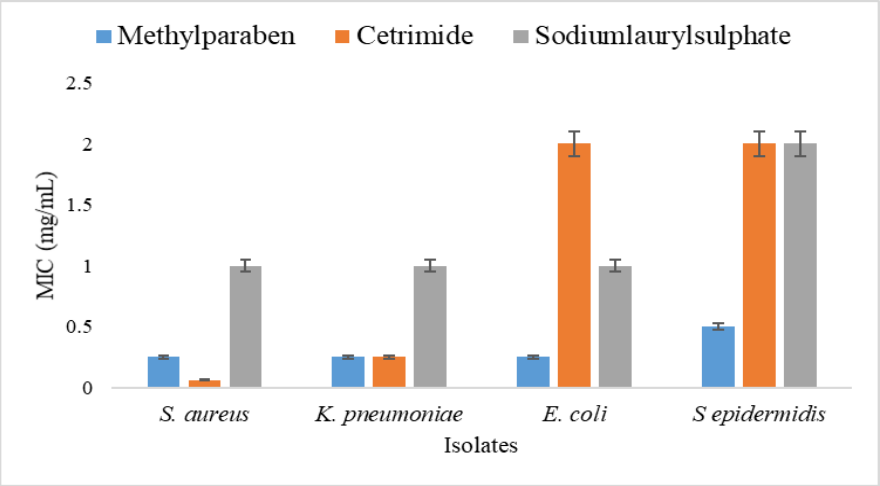

Fig. 2: Individual minimal inhibitory concentrations (MICs) of preservative and surfactants against test organisms.

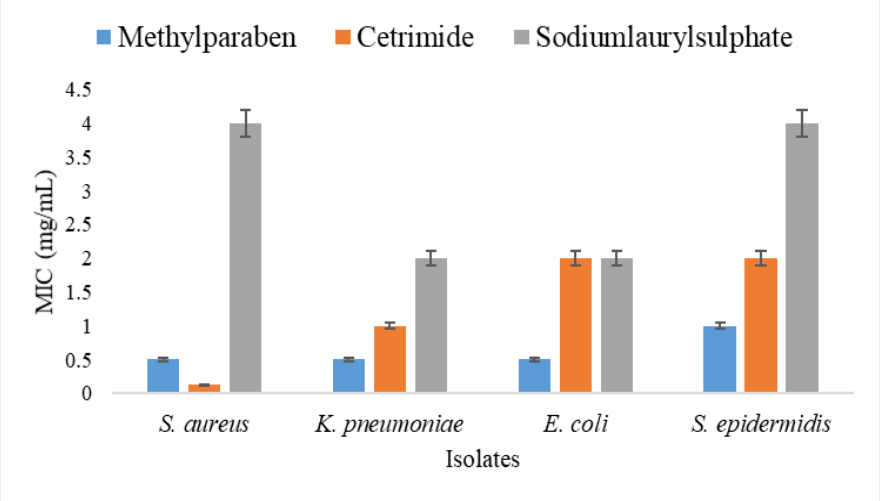

Fig 3: Individual minimum bactericidal concentrations (MBCs) of preservative and surfactants against test organisms.

From the result, Methylparaben has an MIC range of 0.25 - 0.50 mg/mL against the 4 isolates while its bactericidal activity (MBC) was at a concentration range of 0.50 – 1.00 mg/mL. The parabens are reported to be bacteriostatic, but when combined with other agents at low concentration could effectively interfere with the metabolism of microorganisms.[33] As previously reported, the key criteria for antimicrobial preservative selection are the preservative's dose, antimicrobial functionality, and effect on the active ingredient.[34] Cetrimide, a cationic surfactant exhibited its inhibitory action at a concentration of 0.0625 mg/mL against *Staphylococcus aureus*, 0.25 mg/mL against *Klebsiella pneumoniae* and 2.00 mg/mL for *Staphylococcus epidermidis and Escherichia coli*. Previous report indicates that they possess strong bactericidal properties that can be maintained over a wider pH range and in addition be less readily inactivated by proteins.[35] Their mode of action is by altering the permeability of the cell membranes that may lead to cell death.[36] Sodium lauryl sulfate (SLS) (an anionic surfactant) also exhibited its inhibitory action, but not as much as those seen in cationic surfactant. It showed activity at MIC range 1.0 – 2.0 mg/mL and MBC range of 2.0 - 4.0 mg/mL. These figures suggest that sodium dodecyl sulphate has a weak antimicrobial property against the test isolates. This seems to agree with previously report showing that anionic surfactant induce bacterial lysis at high concentrations.[35] Generally, anionic fatty acids are more active against Gram-positive bacteria than against Gram-negative bacteria it their growth.[27, 35]

Evaluation of Combined Effects of Preservative and Surfactant

The results and the inference for the different combinations are as presented in Tables 1 to 8 below. The rationale behind this experiment is that it will result in synergism especially when two antimicrobial agents are acting simultaneously on a uniform microbial population.[10] As previously reported FIC_{index} values < 1 is considered as synergism and its degree of synergism increases as the value tends to Zero. FIC_{index} value of 1 indicates additivity, values greater than 1, but less than 2 represent indifference while values greater than 2 shows antagonism.[26]

Table 1: Combined activities of methylparaben and cetrimide against *S. aureus*

MP : C	MIC (mg/mL) MP	MIC (mg/mL) C	FIC MP	FIC C	FIC index ∑ FIC	Inference
5 : 0	0.25	-	-	-	-	-
4 : 1	0.05	0.003125	0.20	0.05	0.25	Synergism
3 : 2	0.075	0.0125	0.30	0.20	0.50	Synergism
2 : 3	0.025	0.0094	0.10	0.15	0.25	Synergism
1 : 4	0.025	0.025	0.10	0.4	0.50	Synergism
0 : 5	-	0.0625	-	-	-	-

Table 2: Combined activities of methylparaben and SLS against *S. aureus*.

MP : SLS	MIC (mg/mL) MP	MIC (mg/mL) SLS	FIC MP	FIC SLS	FIC index ∑ FIC	Inference
5 : 0	0.25	-	-	-	-	-
4 : 1	0.10	0.10	0.40	0.10	0.50	Synergism
3 : 2	0.0375	0.10	0.15	0.10	0.25	Synergism
2 : 3	0.025	0.15	0.10	0.15	0.25	Synergism
1 : 4	0.025	0.40	0.10	0.40	0.50	Synergism
0 : 5	-	1.00	-	-	-	-

Table 3: Combined activities of methylparaben and cetrimide against *K. pneumonia.*

MP : C	MIC(mg/mL) MP	MIC(mg/mL) C	FIC MP	FIC C	FIC index ∑ FIC	Inference
5 : 0	0.250	-	-	-	-	-
4 : 1	0.10	0.025	0.40	0.10	0.50	Synergism
3 : 2	0.0187	0.0125	0.075	0.050	0.125	Synergism
2 : 3	0.0125	0.0187	0.050	0.075	0.125	Synergism
1 : 4	0.0125	0.050	0.050	0.20	0.25	Synergism
0 : 5	-	0.250	-	-	-	-

Table 4: Combined activities of methylparaben and SLS against *K. pneumonia.*

MP : SLS	MIC (mg/mL) MP	MIC (mg/mL) SLS	FIC MP	FIC SLS	FIC index ∑ FIC	Inference
5 : 0	0.25	-	-	-	-	-
4 : 1	0.05	0.05	0.20	0.05	0.25	Synergism
3 : 2	0.0375	0.10	0.15	0.10	0.25	Synergism
2 : 3	0.05	0.30	0.20	0.30	0.50	Synergism
1 : 4	0.025	0.40	0.10	0.40	0.50	Synergism
0 : 5	-	1.00	-	-	-	-

Table 5: Combined activities of methylparaben and cetrimide against *E. coli.*

MP : C	MIC (mg/mL) MP	MIC (mg/mL) C	FIC MP	FIC C	FIC index ∑ FIC	Inference
5 : 0	0.25	-	-	-	-	-
4 : 1	0.10	0.20	0.40	0.10	0.50	Synergism
3 : 2	0.075	0.40	0.30	0.20	0.50	Synergism
2 : 3	0.10	1.20	0.40	0.60	1.00	Additivity
1 : 4	0.050	1.60	0.20	0.80	1.00	Additivty
0 : 5	-	2.00	-	-	-	-

Table 6: Combined activities of methylparaben and SLS against *E. coli.*

MP : SLS	MIC (mg/mL) MP	MIC (mg/mL) SLS	FIC MP	FIC SLS	FIC index ∑ FIC	Inference
5 : 0	0.25	-	-	-	-	-
4 : 1	0.20	0.20	0.80	0.20	1.00	Additivity
3 : 2	0.075	0.20	0.30	0.20	0.50	Synergism
2 : 3	0.025	0.15	0.10	0.15	0.25	Synergism
1 : 4	0.050	0.80	0.20	0.80	1.00	Additivity
0 : 5	-	1.00	-	-	-	-

Table 7: Combined activities of methylparaben and cetrimide against *S. epidermidis.*

MP : C	MIC (mg/mL) MP	MIC (mg/mL) C	FIC MP	FIC C	FIC index ∑ FIC	Inference
5 : 0	0.50	-	-	-	-	-
4 : 1	0.10	0.10	0.20	0.10	0.30	Synergism
3 : 2	0.01875	0.05	0.40	0.05	0.45	Synergism
2 : 3	0.025	0.15	0.05	0.15	0.20	Synergism
1 : 4	0.05	0.80	0.10	0.80	0.90	Synergism
0 : 5	-	1.00	-	-	-	-

Table 8: Combined activities of methylparaben and SLS against *S. epidermidis.*

MP : SLS	MIC (mg/mL) MP	MIC (mg/mL) SLS	FIC MP	FIC SLS	FIC index ∑ FIC	Inference
5 : 0	0.50	-	-	-	-	-
4 : 1	0.20	0.20	0.40	0.20	0.60	Synergism
3 : 2	0.15	0.40	0.30	0.40	0.70	Synergism
2 : 3	0.10	0.60	0.20	0.60	0.80	Synergism
1 : 4	0.05	0.90	0.10	0.90	1.00	Additivity
0 : 5	-	1.00	-	-	-	-

The combined effect of methylparaben and cetrimide against *S. aureus* (Table 1) showed that synergism occurred in all the ratios (4:1, 3:2, 2:3, 1:4). It also showed synergism in all the ratios when the same preservative is combined with sodium lauryl sulfate (Table 2). Table 3 and 4 presents data obtained for methylparaben in combination with cetrimide and SLS respectively against *K. pneumoniae*. All combinations showed synergism with the highest

activity at 3:2 and 2:3 ratio with methylparaben and cetrimide combination. Table 5 and 6 is the result of Methylparaben-cetrimide and methylparaben-SLS combinations respectively against *E. coli*. Synergism occurred at ratio 4:1 and 3:2 while additivity at occurred at 2:3 and 1:4 ratio for methylparaben-cetrimide combination. Methylparaben-SLS combination, showed synergism at 3:2 and 2:3 while additivity occurred at ratios 4:1 and 1:4. Table 7 and 8 presents the 2 sets of combination against *S. epidermidis.* There was synergism at all ratios for methylparaben-cetrimide combination. For methylparaben-SLS combination, synergism occurred at ratios 4:1, 3:2 and 2:3 and additivity at ratio 1:4.

A comparison of the two surfactants, shows that cationic surfactant combinations (cetrimide) were more effective than the anionic surfactant combinations (SLS). Majority of the combination exhibited synergism, others showed additivity, and none demonstrated antagonism against test organisms. The surfactants seem to increase the antimicrobial activity of the preservative which resulted in the synergism that was demonstrated. This further suggests that the effect of surfactants in the combination reduces the surface tension of the medium thereby increasing the surface activity of the preservative. Reduction in surface tension and interfacial tension would most probably increased the adsorption and uptake of the combinations by the test thereby killing the cells at a faster rate as previously reported.[3] The interaction between surfactants and their target cells could be very complex as previously reported which also results in the formation of secondary structures that are very weak and prone to any agent.[7]

An increased value of synergism observed in combinations of methylparaben and the two surfactants against *S. epidermidis* may be due to the formation of micelles by the surfactants at high concentrations.[27] This seems to agree with previous report that at a higher concentration of the surfactants (that is, concentrations above or equal to its critical micelle concentration), the surface tension becomes saturated and almost constant leading to occlusion of the preservatives in the interior of the micelle with a resultant decrease in the activity of the preservative.[27] In this study, the additivity exhibited by methylparaben-SLS combination should may be attributed to the critical micelle concentration of sodium lauryl sulfate and the fact that sodium lauryl sulfate is a weak antimicrobial agent when compared with cationic surfactants.

CONCLUSION

The present study has demonstrated that pharmaceutical products can be preserved during production, storage and/or during usage by providing unfavourable environments for bacterial and fungal growth. Data obtained indicates that synergistic effects can be achieved when surfactants are used in combination with preservative agents against both gram-negative and gram-positive microorganisms. This can be attributed to the inherent ability of the surfactants to sensitize the bacteria cells in the presence of preservatives by increasing the permeability of the cell membrane against the preservative resulting in cytoplasmic membrane damage.[35, 36] However, there is the need to understand the possible interactions between preservatives and other ingredients of pharmaceutical preparations, in order to select the tolerable dosage. Further testing of these combinations may be required against a wider range of resistant organisms and in experimental models of pharmaceuticals in order to obtain more insight into their mode of action.

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